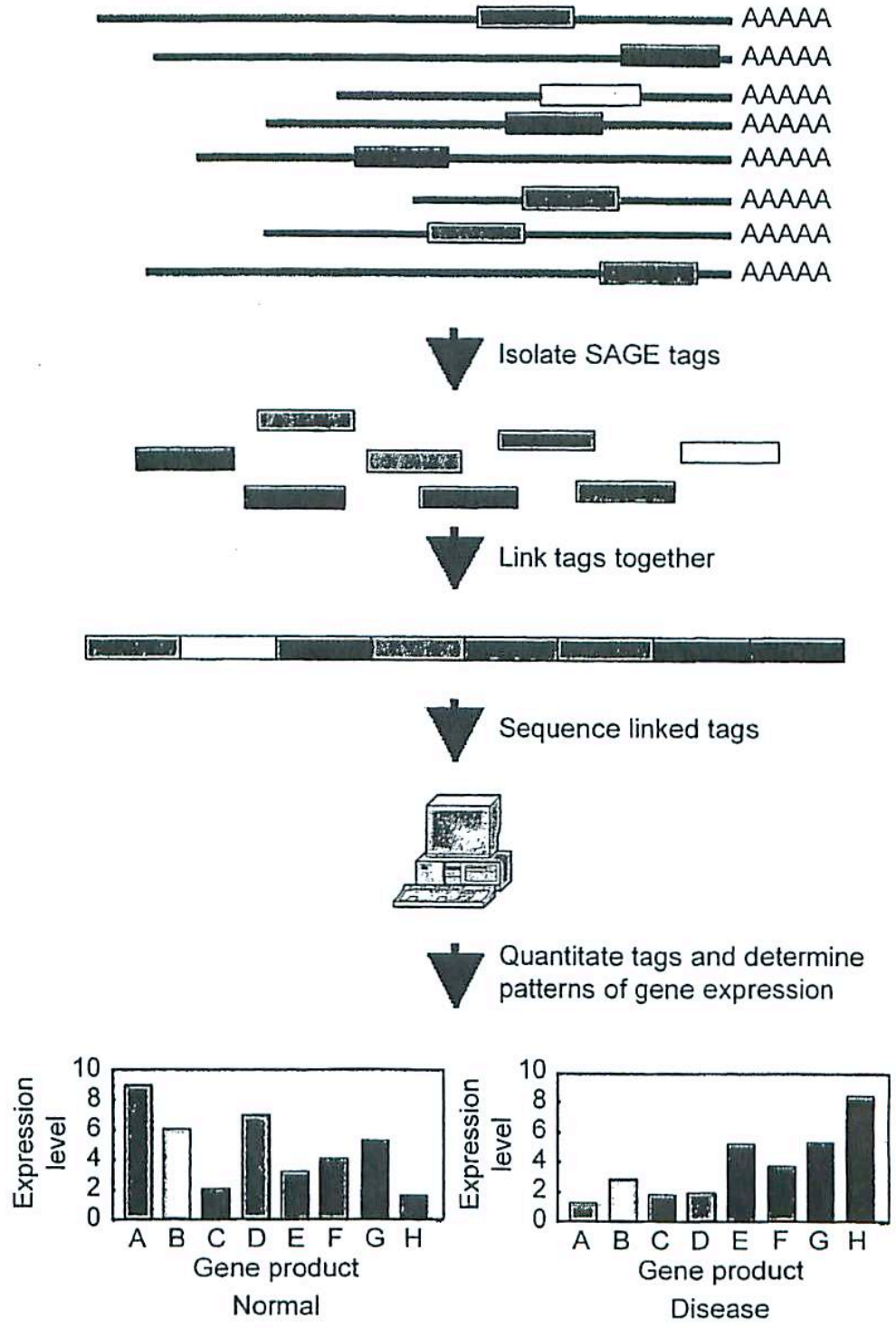

AAAAA
AAAAA
AAAAA
AAAAA
AAAAA
AAAAA
AAAAA
AAAAA
Isolate SAGE tags
Link tags together
Sequence linked tags
Quantitate tags and determine patterns of gene expression
Expression level
10
8
6
4
2
0
A B C D E F G H
Gene product
Normal
Expression level
10
8
6
4
2
0
A B C D E F G H
Gene product
Disease

FIG. 1A

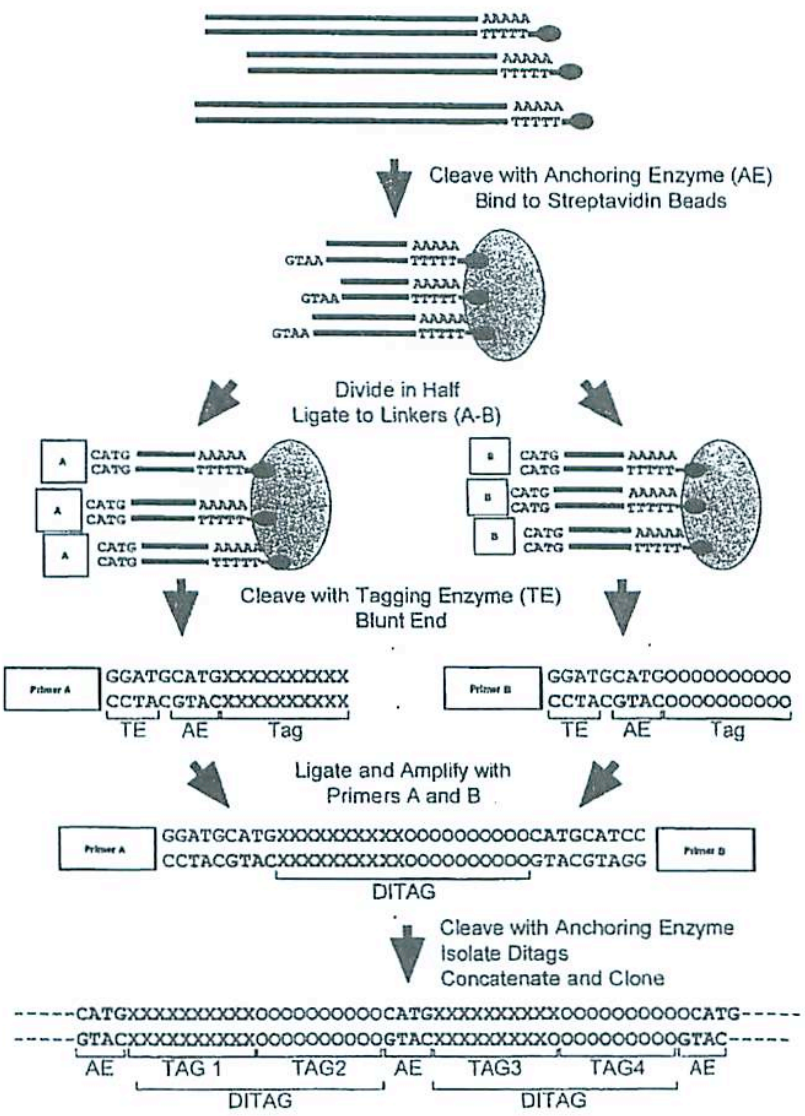

Cleave with Anchoring Enzyme (AE)
Bind to Streptavidin Beads
Divide in Half
Ligate to Linkers (A-B)
Cleave with Tagging Enzyme (TE)
Blunt End
Primer A
Primer B
GGATGCATGXXXXXXXXXX
CCTACGTACXXXXXXXXXX
GGATGCATGOOOOOOOOOO
CCTACGTACOOOOOOOOOO
TE
AE
Tag
Ligate and Amplify with
Primers A and B
GGATGCATGXXXXXXXXXXOOOOOOOOOOCATGCATCC
CCTACGTACXXXXXXXXXXOOOOOOOOOOGTACGTAGG
DITAG
Cleave with Anchoring Enzyme
Isolate Ditags
Concatenate and Clone
-----CATGXXXXXXXXXXOOOOOOOOOOCATGXXXXXXXXXXOOOOOOOOOOCATG-----
-----GTACXXXXXXXXXXOOOOOOOOOOGTACXXXXXXXXXXOOOOOOOOOOGTAC-----
AE
TAG 1
TAG2
AE
TAG3
TAG4
AE
DITAG
DITAG

FIG. 1B

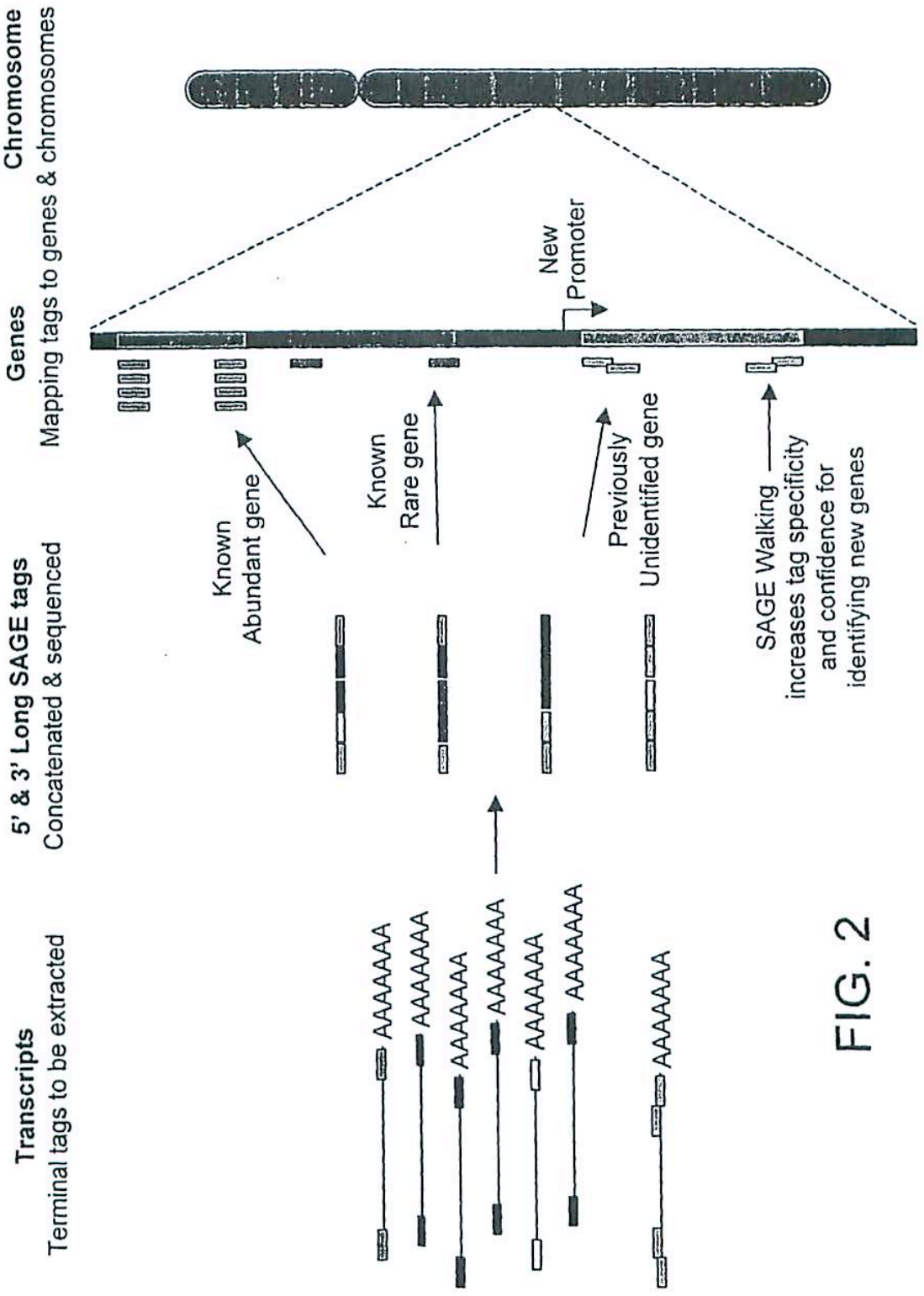
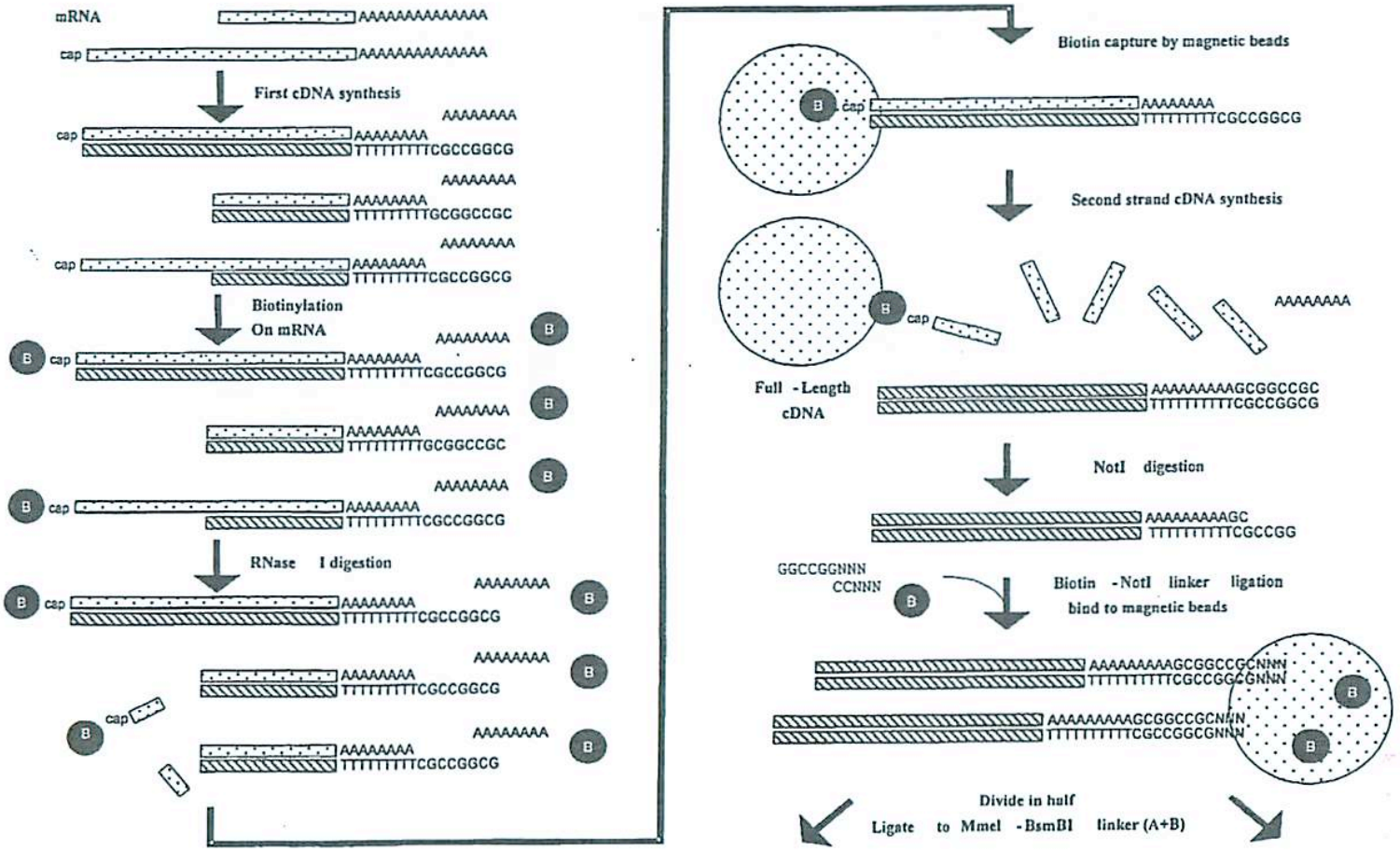
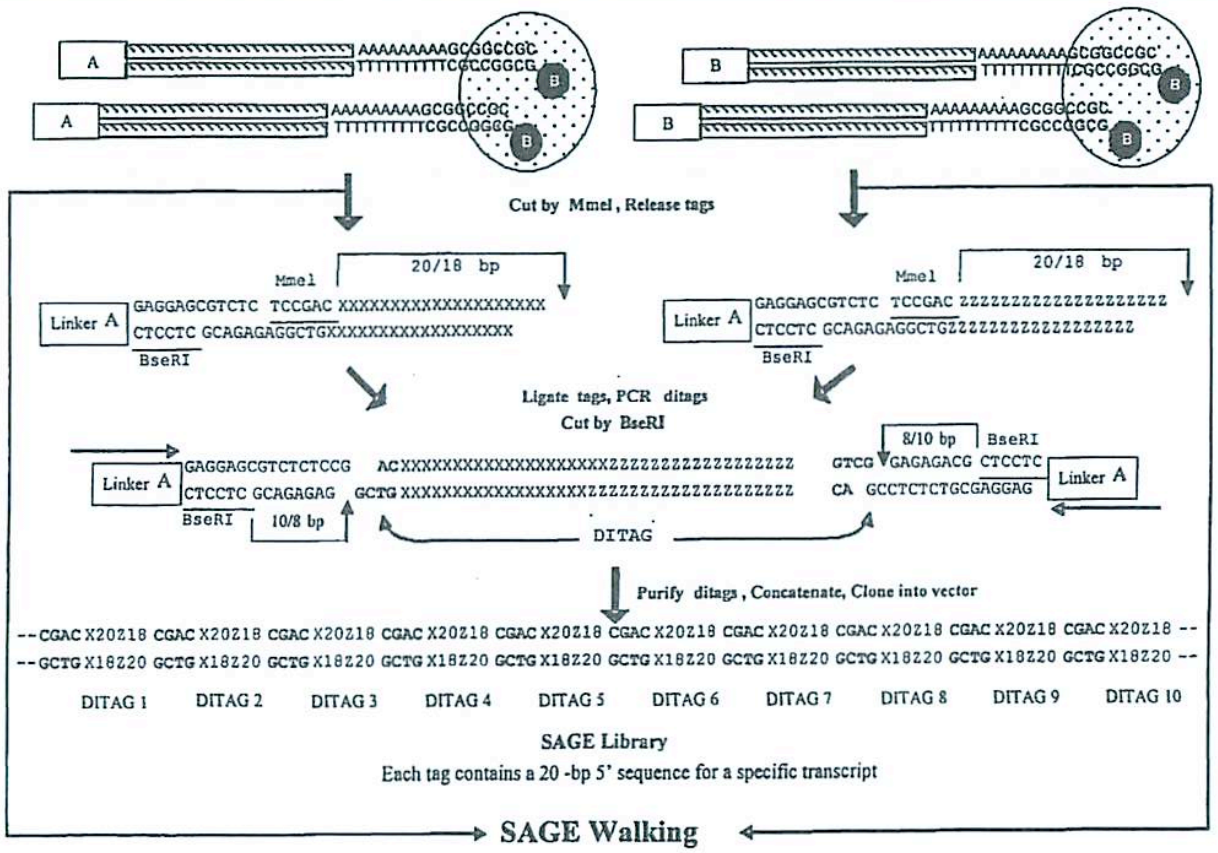

Transcripts
Terminal tags to be extracted
5' & 3' Long SAGE tags
Concatenated & sequenced
Genes
Mapping tags to genes & chromosomes
Chromosome
Known
Abundant gene
Known
Rare gene
New
Promoter
Previously
Unidentified gene
SAGE Walking
increases tag specificity
and confidence for
identifying new genes
AAAAAAA

FIG. 2

FIG. 3

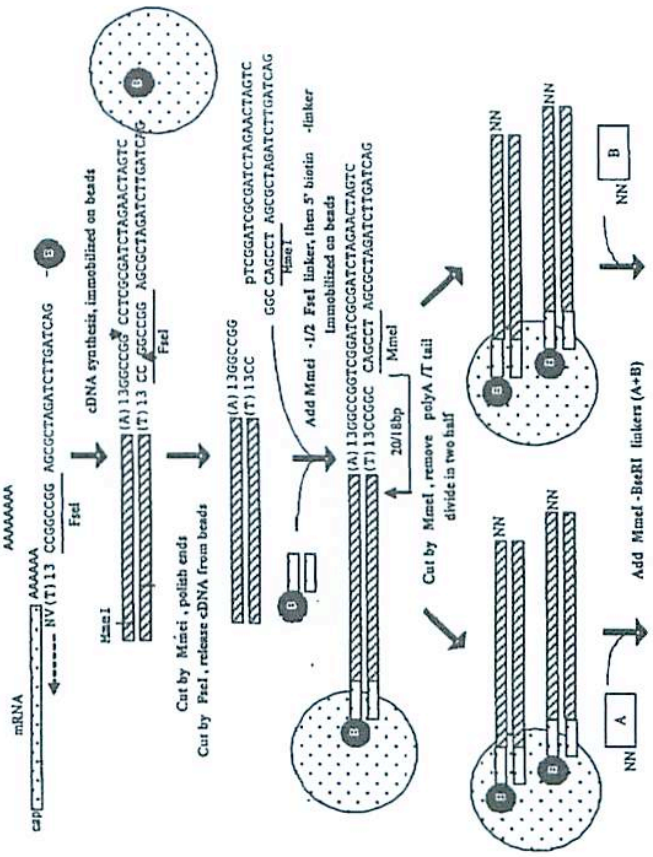

mRNA
AAAAAAAAAAAAAA
cap
First cDNA synthesis
AAAAAAAA
TTTTTTTTCGCCGGCG
TTTTTTTTGCGGCCGC
Biotinylation
On mRNA
B
RNase I digestion
Biotin capture by magnetic beads
Second strand cDNA synthesis
Full - Length
cDNA
AAAAAAAAAGCGGCCGC
TTTTTTTTTCGCCGGCG
NotI digestion
AAAAAAAAAGC
TTTTTTTTTCGCCGG
GGCCGGNNN
CCNNN
Biotin - NotI linker ligation
bind to magnetic beads
Divide in half
Ligate to MmeI - BsmBI linker (A+B)

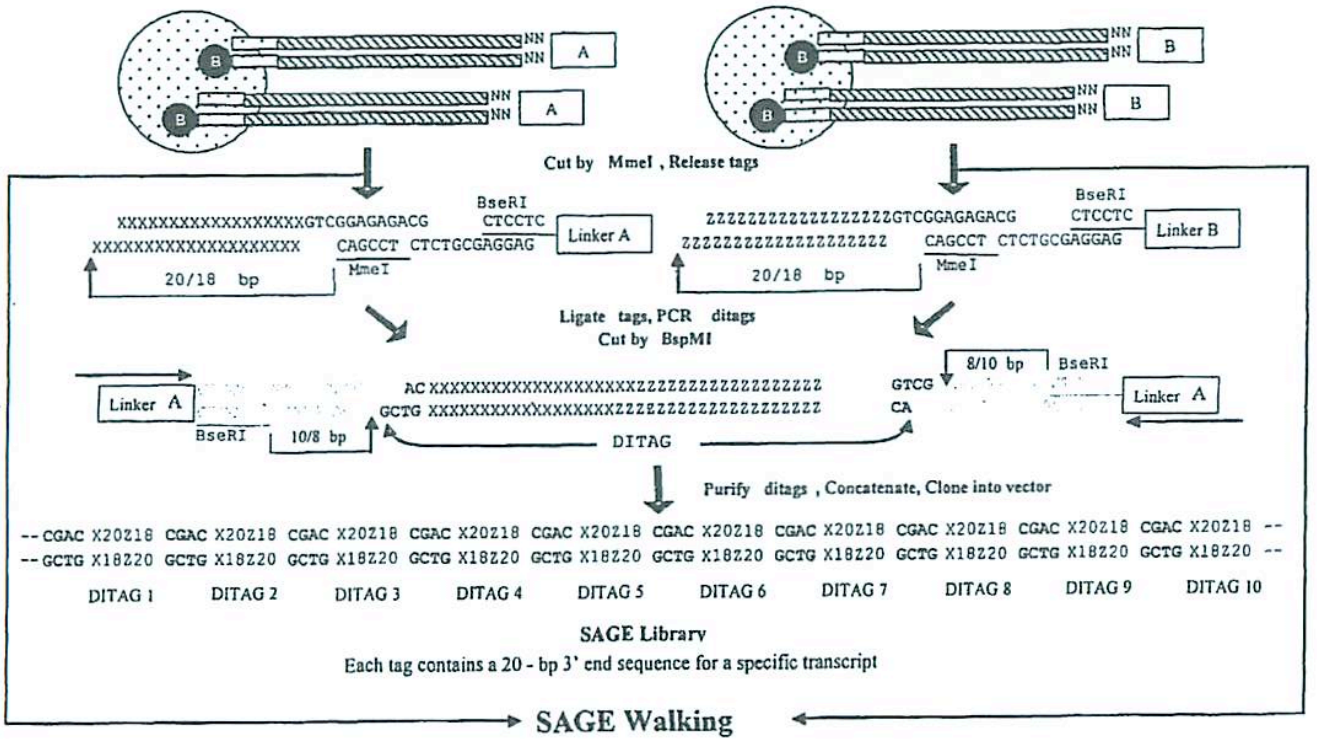


The second round of adding the Mme I-BsmBI linkers, releasing the tags, Ligating and PCR the ditags, removing the arms, purifying ditags, Concatenating and cloning → SAGE Walking Library

FIG. 3 CONT'D

FIG. 4


mRNA
AAAAAAA
cap
NV(T)13
AAAAAA
CCGGCCGG AGCGCTAGATCTTGATCAG
FseI
cDNA synthesis, immobilized on beads
HaeI
(A) 13GGCCGG CCTCGCGATCTAGAACTAGTC
(T) 13 CC GGCCGG AGCGCTAGATCTTGATCAG
FseI
Cut by MaeI, polish ends
Cut by FseI, release cDNA from beads
(A) 13GGCCGG
(T) 13CC
PTCGGATCGCGATCTAGAACTAGTC
GGC CAGCCT AGCGCTAGATCTTGATCAG
MaeI
Add MmeI -1/2 FseI linker, then 5' biotin -linker
Immobilized on beads
(A) 13GGCCGGTCGGATCGCGATCTAGAACTAGTC
(T) 13CCGGC CAGCCT AGCGCTAGATCTTGATCAG
20/18bp
MmeI
Cut by MmeI, remove polyA /T tail
divide in two half
NN
NN
NN
NN
NN
A
Add MmeI -BseRI linkers (A+B)
NN
B



The second round of adding the Mme I-BsmBI linkers, releasing the tags,
Ligating and PCR the ditags, removing the arms, purifying ditags,
Concatenating and cloning → SAGE Walking Library

FIG. 4 CONT'D